Changes of Ribulose Bisphosphate Carboxylase/Oxygenase Content, Ribulose Bisphosphate Concentration, and Photosynthetic Activity during Adaptation of High-CO$_2$ Grown Cells to Low-CO$_2$ Conditions in *Chlorella pyrenoidosa*[1]

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ABSTRACT

Changes of some photosynthetic properties of high-CO$_2$ grown cells of *Chlorella pyrenoidosa* during adaptation to low-CO$_2$ conditions have been investigated. The K_m value of photosynthesis of the high-CO$_2$ grown cells for dissolved inorganic carbon was 3.3 millimolar and decreased to 25 to 30 micromolar within 4 hours after transferring to air. In the presence of saturating CO$_2$ concentrations the photosynthetic activity of the high-CO$_2$ grown cells was 1.5 times as high as that of the low-CO$_2$ grown cells. There was a significant rise of the photosynthetic activity during adaptation of the high-CO$_2$ grown cells to air, followed by a steady decrease. The activity of ribulose 1,5-bisphosphate carboxylase/oxygenase in both the high- and low-CO$_2$ grown cells was close to the photosynthetic activity of the cells. The concentration of ribulose 1,5-bisphosphate (RuBP) was higher in the low-CO$_2$ adapting and low-CO$_2$ grown cells than in the high-CO$_2$ grown cells regardless of the photosynthetic rate. This seems to be due to an increased RuBP regeneration activity during adaptation followed by maintenance of the new higher concentration. The RuBP level always exceeded the concentration of ribulose 1,5-bisphosphate carboxylase/oxygenase RuBP binding sites in both the high- and low-CO$_2$ grown cells at any dissolved inorganic carbon concentration.

To understand the control of photosynthetic CO$_2$ fixation, it is important to know the limiting step(s) of photosynthesis under various environmental conditions. Farquhar *et al.* (6, 7) and von Caemmerer and Farquhar (22) analyzed the regulation of photosynthetic CO$_2$ fixation in C$_3$ plant photosynthesis. In their model (7), photosynthetic CO$_2$ fixation in the presence of saturating light is limited by the activity of Rubisco,[3] when the CO$_2$ concentration is low, while it is limited by the rate of production of chemical energy on thylakoid membranes or ultimately by the rate of RuBP regeneration from PGA, when the CO$_2$ concentration is saturating. Their model has been supported by Badger *et al.* (3), who showed that the concentration of RuBP in bean leaves was much higher than that of the RuBP binding sites of

Rubisco at low CO$_2$ and was decreased to or below the level of the binding sites with increasing atmospheric CO$_2$ concentration. Perchorowicz *et al.* (17), however, obtained results with wheat leaves that were not consistent with Farquhar's model.

A mechanism which seems to regulate the CO$_2$ conditions in the cells occurs in green and blue-green algae (2, 11, 14, 19). Algal cells grown with high-CO$_2$ concentrations seem to lack an effective CO$_2$ concentrating system, but the mechanism to concentrate CO$_2$ in algal cells is induced when they are grown on low-CO$_2$ concentrations such as air (12, 13, 15, 18, 20). The adaptation to low-CO$_2$ conditions is completed a few hours after transferring the cells, grown on high-CO$_2$ concentrations, to air, and is suppressed by protein synthesis inhibitors (12, 13). While several studies report the changes in photosynthetic characteristics (12, 13, 15, 19, 21) or changes in carbonic anhydrase levels and Rubisco content (8, 18, 20, 21, 23, 25) that occur when cells are transferred from high-CO$_2$ to low-CO$_2$ conditions, there has been little information on the regulation of the photosynthetic carbon reduction cycle during this adaptation.

In the present paper, we determined Rubisco content, RuBP and PGA concentrations, and photosynthetic activity during adaptation of high-CO$_2$ grown cells of *Chlorella pyrenoidosa* to growth on air. Based on these results and the apparent ability for RuBP regeneration in high- and low-CO$_2$ grown cells of *Chlorella* during photosynthesis, some comments are offered on the regulation of photosynthesis in *Chlorella*.

MATERIALS AND METHODS

Organism and Culturing. *Chlorella pyrenoidosa* Chick (UTEX 252) was grown in Allen's medium buffered with 50 mM glycylglycine buffer at pH 8.0 at a light intensity of 200 to 250 μE m^{-2} s^{-1} and 25°C (25). The culture was bubbled with sterile 1% CO$_2$ in air or air. The cells, in exponential growth, were collected by centrifugation at 1000g for 3 min at room temperature (20–25°C) and used for experiments immediately.

Measurement of Photosynthetic O$_2$ Evolution. The O$_2$ evolution measurement method of Miller *et al.* (15) was used, unless otherwise stated. *Chlorella* cells were collected by centrifugation at 10,000g for a few seconds at room temperature, washed with 50 mM glycylglycine buffer (pH 8.0) containing low dissolved CO$_2$, suspended in the same buffer, and bubbled with CO$_2$-free N$_2$ gas until the O$_2$ concentration decreased to less than 5 μM. After the O$_2$ compensation point, where endogenous DIC in the medium or as a pool in the cells was depleted and photosynthetic O$_2$ evolution and respiratory O$_2$ uptake was balanced, was attained at a light intensity of 250 μE m^{-2} s^{-1}, photosynthetic O$_2$ evolution was measured with a Clark-type O$_2$ electrode (Hansa-

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[3] Abbreviations: RuBP, ribulose 1,5-bisphosphate; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; PGA, 3-phosphoglycerate; DIC, dissolved inorganic carbon.

tech Ltd., Kings Lynn, Norfolk, U.K.) at 25°C in a reaction mixture (1 ml) composed of 50 mM glycylglycine buffer (pH 8.0), 250 units of carbonic anhydrase, and various concentrations of sodium bicarbonate. The light intensity was 250 μE m^{-2} s^{-1}.

Determination of RuBP and PGA. *Chlorella* cells, photosynthesizing under illumination, were killed by drawing 9 ml of the cell suspension into an illuminated disposable syringe which contained 1 ml of 4.5 M HClO$_4$. If the syringe was not illuminated during killing, the measured RuBP content was decreased by 50%. The killed cells were cooled promptly in ice and sonicated at 0°C for 1 min. The sonicate was centrifuged at 10,000g for 10 min to remove denatured cell protein and debris. The supernatant was neutralized to pH 6.0 to 7.0 with 5 and 0.5 M KOH, and stored in ice for 30 min. The resulting KClO$_4$ was removed by centrifugation at 10,000g for 5 min and the supernatant was lyophilized. The lyophilized metabolites were dissolved in 0.5 ml of 50 mM Tris-HCl buffer (pH 8.0) at 0°C, and KClO$_4$ was again removed by centrifugation at 10,000g for 10 s. The supernatant was used for determinations of RuBP and PGA.

RuBP in the extract was determined by measuring acid stable ^{14}C after the reaction of the extracted RuBP and NaH^{14}CO$_3$, the specific radioactivity of which had been determined, for 1 h at 25°C (3) in the presence of fully activated Rubisco purified from spinach to an electrophoretically homogeneous state (25). PGA was determined by measuring the absorbancy change of NADH in the conversion of PGA to glycerol-P in the presence of phosphoglycerate kinase, glyceraldehyde 3-P dehydrogenase (NAD$^+$), triose-P isomerase, and glycerol-P dehydrogenase (NAD$^+$) (3).

Recoveries of authentic RuBP and PGA added to the *Chlorella* cells were 50.7 ± 3.2% (n = 3) and 92.2 ± 2.2% (n = 3), respectively. The concentrations of these metabolites reported here have been corrected for the above recoveries.

Determination of Rubisco Content and Activity. The concentration of RuBP binding sites of Rubisco in *Chlorella* cell extracts prepared as reported previously (25) was determined by the [^{14}C] carboxypentitol bisphosphate-polyethylene glycol method (25). Rubisco content was calculated by dividing the quantity of binding sites by the mol wt of one large plus one small subunit. Cell disruption for the assay of the activity and the assay followed the previous methods.

DIC and Chl Measurement. DIC was determined by the method of Miller *et al.* (15). Chl was quantified according to Arnon (1).

RESULTS

RuBP and PGA Content of Low-CO$_2$ Grown *Chlorella*. The photosynthetic O$_2$ evolution and amounts of RuBP and PGA in low-CO$_2$ grown cells of *Chlorella* are shown in Figure 1 when 1 mM sodium bicarbonate was added to the cells after they had reached the O$_2$ compensation point. Photosynthetic O$_2$ evolution showed a lag phase for about 3 min before it reached its maximum activity. RuBP concentration was 120 nmol mg^{-1} Chl at the O$_2$ compensation point, decreased to 35 nmol mg^{-1} Chl 15 s after the addition of bicarbonate, and thereafter was constant at 37 nmol mg^{-1} Chl. The PGA level was 48 nmol mg^{-1} Chl at the O$_2$ compensation point, and addition of bicarbonate caused a sudden increase to 120 nmol mg^{-1} Chl. This pattern of changes in RuBP and PGA is expected from the operation of the photosynthetic carbon reduction cycle in *Chlorella* photosynthesis and indicates that the method of determining the levels of these metabolites was adequate.

Changes of Photosynthetic Properties of High-CO$_2$ Grown Cells of *Chlorella* during Adaptation to Low-CO$_2$ Conditions. Except for changes in the K_m (DIC) (2, 12, 13, 15, 18, 20) and carbonic anhydrase levels (8, 18, 20), changes in other photosynthetic properties have not been reported for high-CO$_2$ grown
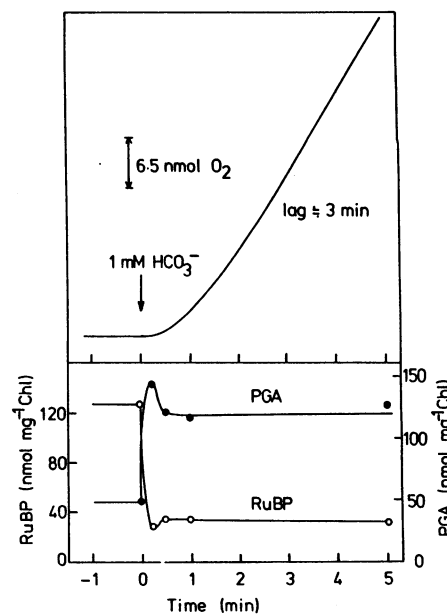

FIG. 1. Changes of RuBP and PGA levels and photosynthetic activity in low-CO$_2$ grown *Chlorella* after addition of bicarbonate to cells at O$_2$ compensation point attained by bubbling with CO$_2$-free air. The photosynthetic activity was determined by an O$_2$ electrode. For determination of the concentrations of RuBP and PGA, the *Chlorella* cells were incubated under the same conditions as for photosynthetic O$_2$ evolution, except that the incubation volume was 5 ml. Chl concentration was 15 μg ml^{-1}. O$_2$ concentration was 21%. The photosynthetic rate was 85 μmol mg^{-1} Chl h^{-1} after a lag period. The figure shows the results from a single experiment that was representative of three separate experiments.

algal cells adapting to low-CO$_2$ conditions. Figure 2 shows changes of photosynthetic activity in the presence of a saturating concentration of DIC (30 mM), K_m (DIC) values of *Chlorella* cells for photosynthesis, intracellular concentrations of RuBP and PGA, and DIC in the medium, when the high-CO$_2$ grown cells of *Chlorella* were bubbled with 1% CO$_2$ in air for 2.5 h in the light, transferred to new 50 mM glycylglycine buffer (pH 8.0), and bubbled with CO$_2$-free air for 30 min in the dark, and then bubbled with air and illuminated at 250 μE m^{-2} s^{-1}. The response of photosynthetic rate to increasing amounts of DIC in high-CO$_2$ grown and low-CO$_2$ grown *Chlorella* and in the high-CO$_2$ grown cells adapted for 2 h to air is fully shown in Figure 3. The high-CO$_2$ grown cells had a maximum photosynthetic activity of 170 μmol mg^{-1} Chl h^{-1}. The maximum activity increased to 220 μmol mg^{-1} Chl h^{-1} 2 h after transfer to air, and then gradually decreased to 120 μmol mg^{-1} Chl h^{-1}. The K_m value of the high-CO$_2$ grown cells for DIC was 3.3 mM and decreased to 25 to 30 μM, which was the value of the low-CO$_2$ grown cells, 2 to 3 h after the transfer.

DIC in the incubation medium was 6.74 mM when the culture was bubbled with 1% CO$_2$ in air, and decreased to 0.18 mM when CO$_2$-free air was bubbled through new glycylglycine buffer in the dark for 30 min. Changing the flushing gas to air and illumination of the cell suspension caused a sudden increase of DIC in the incubation medium to 0.5 mM due to dissolution of air CO$_2$ to the medium and inefficiency of the high-CO$_2$ grown cells of *Chlorella* to photosynthesize at these low DIC concentrations. As the affinity of the *Chlorella* cells for DIC increased, the DIC in the medium gradually decreased to 80 μM and was constant thereafter.

The concentration of RuBP in the high-CO$_2$ grown cells in 1% CO$_2$ was 30 to 60 nmol mg^{-1} Chl. The concentration decreased to 10 nmol mg^{-1} Chl in the dark. In the light 30 min after transferring to air, the concentration had increased to 126 nmol
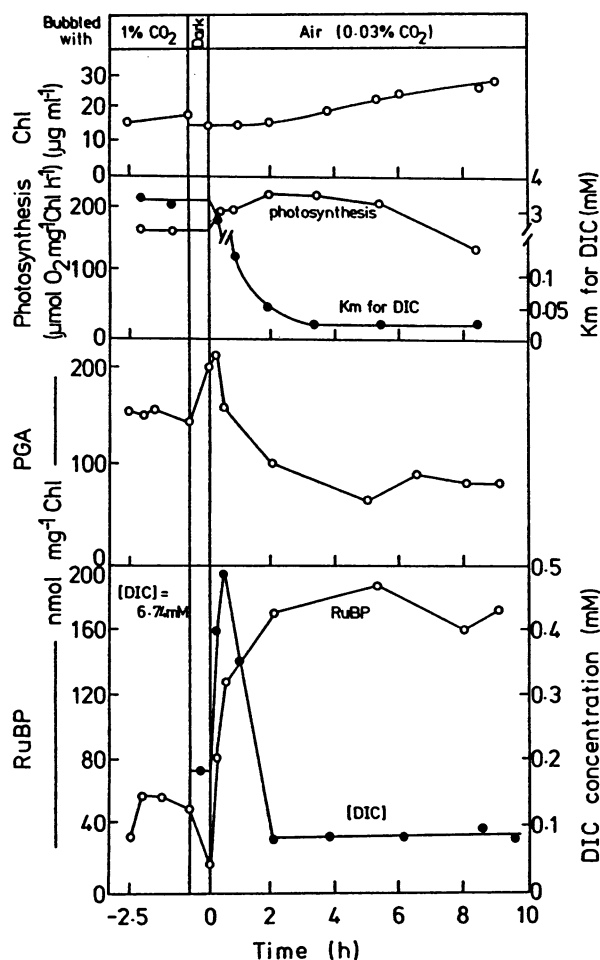
FIG. 2. Changes of photosynthetic properties and RuBP and PGA in high-CO₂ grown cells transferred to air. The incubation mixture was bubbled with 1% CO₂ in air in the light, CO₂-free air in the dark and air at the rate of 2 L min⁻¹ per 400 ml of the mixture. The light path of the plexiglass incubation vessel was 1.2 cm. The initial Chl density was 15 μg ml⁻¹ and the light intensity was 250 μE m⁻² s⁻¹. Samples were removed at the indicated times for the determination of RuBP, PGA, photosynthetic rate in the presence of 30 mM sodium bicarbonate and 250 units of carbonic anhydrase at 250 μE m⁻² s⁻¹ and 25°C, and the K_m(DIC) for photosynthesis.
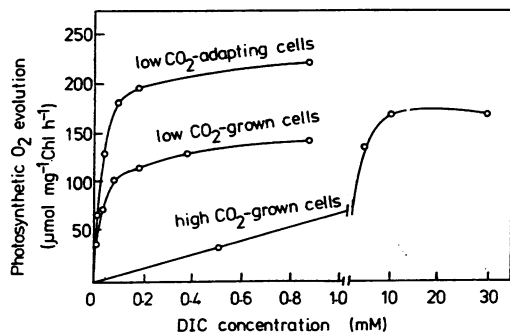

FIG. 3. Photosynthetic O₂ evolution activities of the high-CO₂ grown, low-CO₂ adapting, and low-CO₂ grown cells. The low-CO₂ adapting cells are the cells adapted for 2 h to air in Figure 2.

mg⁻¹ Chl and then continued to increase to a steady state of 170 nmol mg⁻¹ Chl. PGA level was twice as high in the high-CO₂ grown cells in 1% CO₂ as in the low-CO₂ adapted cells in air.

In Figure 2, the K_m value of the high-CO₂ grown cells for DIC

decreased from 3.3 mM to 25 to 30 μM during adaptation of the cells to air. The ratio of [DIC] in the medium to K_m(DIC) was calculated to be 2.0 for the high-CO₂ grown cells in 1% CO₂ and 3.2 for the low-CO₂ adapting cells in air. This implies that the consumption rate of RuBP by the low-CO₂ adapting cells at 80 μM DIC was higher than that of the high-CO₂ grown cells at 6.74 mM DIC, and that the high-CO₂ grown cells should contain higher RuBP levels than the low-CO₂ adapting cells at these respective DIC concentrations, if the rate of regeneration of RuBP was the same in both cell types.

We have calculated the rate of RuBP consumption by Rubisco, namely the rate of photosynthetic CO₂ fixation, at the time when the cells were sampled by resolving Michaelis-Menten equation with the actual values of the [DIC] in the medium, the K_m(DIC) values of the cells and the maximum activities of photosynthesis at that time, all obtained from Figure 2. The calculation assumed that the regeneration rate of RuBP was constant throughout the experiments in Figure 2. These calculations (Fig. 4) showed that the RuBP concentration of the low-CO₂ adapting cells at 80 to 90 μM DIC should have been similar to that of the high-CO₂ grown cells at 6.74 mM DIC because the rate of utilization was assumed to be the same. However, this was not the case, as can be seen in Figure 2. The RuBP levels of the low-CO₂ adapting cells were much higher than those of the high-CO₂ grown cells, indicating that the former cells had a higher efficiency of RuBP regeneration and maintained higher levels of RuBP than the latter.

To further check this observation, the RuBP levels in both cell types were plotted against [DIC]/(K_m of photosynthesis for DIC) (Fig. 5). This kind of plotting is useful for comparing the rates of RuBP consumption between the cells having different K_m values for DIC; [DIC]/K_m for DIC approximates the extent of the attainment of the rate of photosynthesis or RuBP consumption to its maximum. In the high-CO₂ grown cells, the RuBP level was about 100 nmol mg⁻¹ Chl below a ratio of 0.2 of [DIC] /K_m and about 200 nmol mg⁻¹ Chl at a ratio of 0.8 of [DIC]/K_m. Above this point, the level was constant at about 50 nmol mg⁻¹ Chl. The RuBP concentration of the low-CO₂ adapting cells, on the contrary, was 250 to 350 nmol mg⁻¹ Chl at a ratio of 3 of [DIC]/K_m, and about 100 nmol mg⁻¹ Chl when the ratio of [DIC] /K_m was over 4.

In Table I are shown the concentration of RuBP binding sites of Rubisco, the Rubisco content and activity of Rubisco in the high-CO₂ grown, low-CO₂ adapting, and low-CO₂ grown cells of *Chlorella*. The level of RuBP binding sites was estimated to be 22 nmol mg⁻¹ Chl in the high-CO₂ grown cells which corresponded to 1.55 mg Rubisco mg⁻¹ Chl. The Rubisco activity of the high-CO₂ grown cells was 174 μmol mg⁻¹ Chl h⁻¹. These values of the low-CO₂ grown cells were 11 nmol mg⁻¹ Chl, 0.77
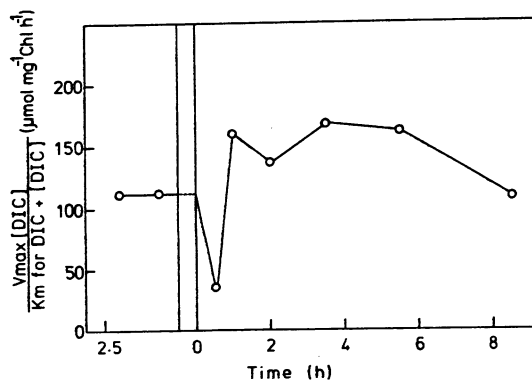

FIG. 4. Calculation of the expected rates of RuBP utilization in the course of adaptation of high-CO₂ cells to low-CO₂ conditions. K_m for DIC, [DIC] and V_{max} were from Figure 2.
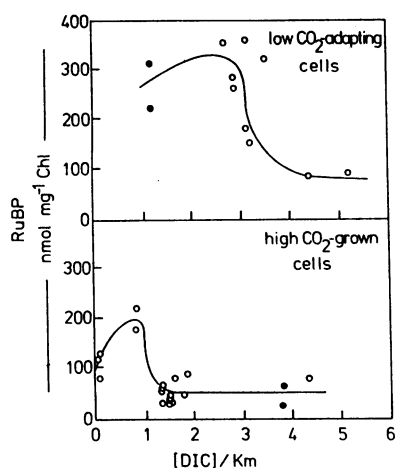
FIG. 5. RuBP levels of the high- and low-CO_2 grown cells plotted against [DIC]/K_m. Data were obtained from several experiments similar to Figure 2. The RuBP levels for the high-CO_2 grown cells were from the incubation period between −2.5 and −0.5 h and those for the low-CO_2 adapting from incubation periods between 3 and 9 h. DIC concentration and K_m were determined in each individual experiment. Each point is the average of two measurements with a variation of less than 5%. Light intensities: O, 250 μE m^{-2} s^{-1}; ●, 500 μE m^{-2} s^{-1}.

mg mg^{-1} Chl, and 116 μmol mg^{-1} Chl h^{-1}, respectively. Adaptation to air for 4 h caused a slight increase in Rubisco content and activity. *Chlorella* cells adapted to air for 9 h showed intermediate values between the two cell types, even though the K_m (DIC) indicated that adaptation to low-CO_2 conditions was completed within 3 h after transfer to a low-CO_2 concentration (Fig. 2). There was seen a difference in specific activity of Rubisco between high-CO_2 grown or low-Co_2 adapting *Chlorella* and low-CO_2 grown *Chlorella*.

Relationship between the RuBP Level and Photosynthetic Activity in High-CO_2 and Low-CO_2 Grown Cells. From these adaptation experiments, it was suggested that the steady state concentration of RuBP during photosynthesis was quite different between high-CO_2 and low-CO_2 grown cells of *Chlorella*. In order to confirm this, the RuBP concentrations and the photosynthetic activities were determined in both cell types at various DIC concentrations. Since photosynthetic O_2 evolution required a half to a few minutes to reach full activity after addition of sodium bicarbonate to the cells at the O_2 compensation point (Fig. 1), the cells were killed only after O_2 evolution was linear at each individual DIC concentration. The cells were killed by adding $HClO_4$ to a final concentration of 0.45 M directly to the cell suspension in a small vial with a serum cap and the acidified suspension was left at the same light intensity (250 μE m^{-2} s^{-1}) as during previous photosynthesis until the cells turned brown. Throughout the incubation and killing, the suspension was stirred with a magnetic stirrer.

The RuBP levels were plotted against the rates of photosyn-

thetic O_2 evolution at various given DIC concentrations up to 30 mM in the high-CO_2 grown cells and 1 mM in the low-CO_2 grown cells (Fig. 6). This type of plot is very useful for comparing RuBP levels in both cell types at the same RuBP utilization rates. The RuBP concentration, in the low-CO_2 grown cells, showed a peak of 180 nmol mg^{-1} Chl (which occurred at the K_m concentration of DIC) and declined to 40 to 120 nmol mg^{-1} Chl at [DIC] which gave rise to the maximum photosynthetic activity. The reason for this large variation is not known. On the other hand, the RuBP level of the high-CO_2 grown cells was 110 nmol mg^{-1} Chl at the O_2 compensation point, and decreased to about 40 nmol mg^{-1} Chl with increasing concentrations of DIC.

DISCUSSION

The expected changes in the amounts of RuBP and PGA for C_3 photosynthesis (24) were obtained in low-CO_2 grown *Chlorella* when bicarbonate was supplied to the cells (Fig. 1). But when high-CO_2 grown cells were transferred to low-CO_2 conditions, the changes in the amount of RuBP were not those that we expected (Figs. 2 and 4). Immediately after the transfer of the high-CO_2 grown cells to low-CO_2 conditions in the light, the rate of photosynthesis in the cells would be small (Fig. 3). During this period the [DIC] in the medium increased (due to dissolution of air CO_2 to the medium and respiration) and the RuBP concentration increased, an observation consistent with a lack of CO_2 in the cells (Fig. 2). After 3 h, as observed by others (18, 20), the cells had adapted to low-CO_2 conditions and the K_m (DIC) for photosynthesis had decreased from 3.3 mM to 50 μM. High rates of photosynthesis would now occur in the cells at the [DIC] of the medium (Figs. 2 and 3) but the RuBP level did not decrease.

This unexpected maintenance of high RuBP levels, when the cells were photosynthesizing at high rates, was investigated further in high-CO_2 grown cells, in such cells adapting to low-CO_2 conditions and in low-CO_2 grown cells. It was found that for similar calculated (Fig. 5) or actual (Fig. 6) rates of photosynthesis the steady state level of RuBP was much higher in low-CO_2 grown cells than in high-CO_2 grown cells. These observed results suggest that the RuBP regeneration rate had increased when high-CO_2 grown cells were transferred to low-CO_2 conditions (Fig. 2) and that the regulation of RuBP levels had now changed so that higher concentrations of RuBP were maintained in the cells (Figs. 5 and 6). The explanation or mechanism for these changes are not fully apparent at the present time. But the catalytic reaction of Rubisco is a two substrate reaction that proceeds in a random mechanism (10). With this mechanism, a decrease of the concentration of one substrate greatly increases the apparent K_m of the enzyme for the other substrate (5). The elevated level of RuBP in low-CO_2 adapted cells may allow Rubisco to function at full activity when the CO_2 concentration has been increased by the development of the CO_2 concentrating mechanism.

When the high-CO_2 grown cells were adapting to low-CO_2 conditions, there were also complex changes in the Rubisco content (Table I). During the first 4 h, the Rubisco content

Table I. *Changes of Rubisco Content and Activity during Adaptation of High-CO_2 Grown Chlorella Cells to Air*

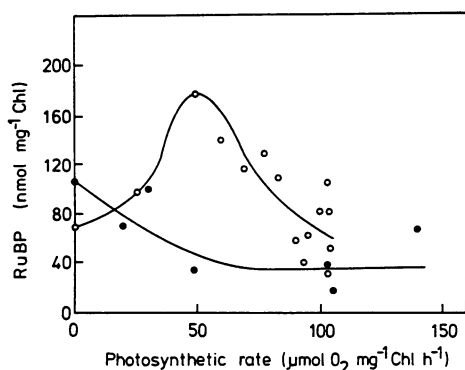| *Chlorella* cells	Concentration of RuBP Binding Sites	Rubisco Content	Rubisco Activity	Rubisco Specific Activity
	nmol mg^{-1} Chl	*mg mg^{-1} Chl*	*μmol mg^{-1} Chl h^{-1}*	*μmol mg^{-1} Rubisco min^{-1}*
High-CO_2 grown	22	1.55	174.3	1.87
Low-CO_2 adapting				
4 h-adapted	28	1.95	190.4	1.63
9 h-adapted	13	0.94	145.9	2.59
Low-CO_2 grown	11	0.77	116.6	2.52

FIG. 6. Relationship between RuBP level and photosynthetic activity in the high- (●) and low-CO_2 (O) grown cells. The K_m values for photosynthesis of the high- and low-CO_2 grown cells at 21% O_2 for [DIC] were 3.3 mM and 11.2 μM, respectively. Other conditions were as follows: Chl concentration, 15 μg ml^{-1}; O_2 concentration, 21%; [DIC], as required for indicated photosynthetic rate.

increased by 27% and this increase was reflected by a 30% increase in the maximum photosynthetic rate (Fig. 2). Thereafter, the Rubisco content declined so that the content of cells fully adapted to low-CO_2 conditions was only 50% of that observed in the high-CO_2 grown cells (Table I). The reason why the specific activity of Rubisco was increased during the adaptation is not clear at present. The Rubisco content *Scenedesmus* and *Anabaena* was also influenced in a similar manner by the [DIC] during growth (25). The maximum photosynthetic rate of the cells also decreased (Figs. 2 and 3) but the decrease was smaller than the decrease in Rubisco content. This apparent increase in the efficiency of CO_2 fixation by Rubisco may be due to the development of the CO_2 concentrating mechanism and carbonic anhydrase. It is well known that carbonic anhydrase is induced in high-CO_2 grown cells adapted to low-CO_2 conditions (4, 8, 18, 20). Since carbonic anhydrase enhances the activity of Rubisco by supplying CO_2 from bicarbonate in the cells (16), the presence of this enzyme, accompanied by high CO_2 content in the cells, may give rise to higher maximum rates of CO_2 fixation per unit of Rubisco.

The simultaneous determination of Rubisco content and RuBP levels allows one to consider limiting steps in *Chlorella* photosynthesis. The theory of Farquhar *et al.* (6, 7) proposes that photosynthetic CO_2 fixation is limited by the low affinity of Rubisco under low-CO_2 conditions and is limited by RuBP concentration under saturating CO_2 conditions. In *Chlorella*, however, the concentration of RuBP never decreased below the number of RuBP binding sites but was always at least double the concentration of the binding sites. This would suggest that under saturating light, *Chlorella* photosynthesis is not limited by the amount of RuBP at any DIC concentration. At saturating DIC concentration it would seem that Rubisco is fully activated and the maximum photosynthetic rate of *Chlorella* is determined by the amount of Rubisco in the cells.

von Caemmerer *et al.* (23) reported that the RuBP level in *Chlamydomonas* was below the concentration of RuBP binding sites in the presence of high DIC concentrations. The measured levels of RuBP in their experiments, however, may be low because they did not illuminate the syringe in which the cells were killed and we have found that a lack of illumination during this step resulted in extractable RuBP levels of about one-half that obtained with an illuminated syringe. Badger *et al.* (3) also reported that the RuBP concentration was less than the concentration of RuBP binding sites in bean leaves under saturating

CO_2 concentrations. However, they compared the RuBP concentration to the concentration of RuBP binding sites reported for spinach (9) but the concentration of binding sites in bean may be lower than in spinach. Certainly, in *Chlorella*, the concentration of RuBP does not appear to ever limit photosynthesis but additional results are required to determine the frequency and distribution of this situation.

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